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Intracellular Spatial Control of Fluorescent Magnetic Nanoparticles

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This communication describes the intracellular manipulation of fluorescent magnetic nanoparticles by magnetic force. Quantum dots (QDs) as fluorescent probes have found increased applications for cell labeling, tracking of cell migration, and in vivo imaging.[1] Magnetic nanoparticles also show many advantages in biological applications (e.g., as a new type of magnetic resonance imaging contrast agent[2]). One unique feature of magnetic nanoparticles is to respond well to magnetic control, which has led to several successful applications, including biological separation, protein purification, bacteria detection, and drug delivery.[3] The recent development of multifunctional nanostructures has attracted increased attention because of their advantageous properties.[4] The combination of quantum dots and magnetic nanoparticles has led to new applications in biological systems.[5] Such fluorescent magnetic nanoparticles bear two attractive features, fluorescence and superparamagnetism, that allow their intracellular movements to be controlled using magnetic force and to be monitored using a fluorescent microscope. These features could lead to effective ways to probe specific functions of bioactive molecules in localized domains or compartments of living cells without disturbing other parts of the cell. For example, fluorescent magnetic nanoparticles could act as a vehicle to deliver activators or inhibitors specifically to the apical or basolateral domain in epithelial cells under a magnetic field, and a fluorescent microscope could then detect the positions of the particles. Toward this goal, we report on the demonstration of intracellular manipulation of fluorescent magnetic Fe_3O_4−CdSe nanoparticles using a small magnet.

As shown in Scheme 1, the nanoparticles or their clusters exhibit random motion in a cell without a magnetic field. Upon the application of the magnetic field, a bias originating from the magnetic field causes the nanoparticles to aggregate to one side of the cell. The strategy demonstrated here will help to probe the functions of polarized (asymmetric) cells, such as epithelial cells that have structurally distinct apical and basolateral domains or neuron cells that have distinctive cell bodies, to provide an effective way for specifically manipulating the functions of proteins in a desired intracellular location, and ultimately to introduce a new approach to understanding basic cellular processes and functions.

Using 4-nm Fe_3O_4 nanoparticles[6] as seeds, we adopted the procedure described by Peng et al.[7] to grow CdSe quantum dots on the Fe_3O_4 nanoparticles and obtained Fe_3O_4−CdSe heterodimers. As shown in the high-resolution transmission electron microscope (HRTEM) image (Figure 1A), the darker regions correspond to Fe_3O_4 and the lighter ones to CdSe in the heterodimer. The selected area electron diffraction pattern (EDP) of the Fe_3O_4−CdSe nanoparticles (Figure 1B) displays the rings that originate from the zinc blende phase of CdSe ([111], [220], [311], and [400] planes) and the face center cubic (fcc) phase of Fe_3O_4 ([220], [311], [400], and [440] planes). The energy dispersive X-ray spectrometer (EDS) analysis[8] of the heterodimers further confirms that the darker regions mainly consist of Fe and the lighter ones of Cd and Se.

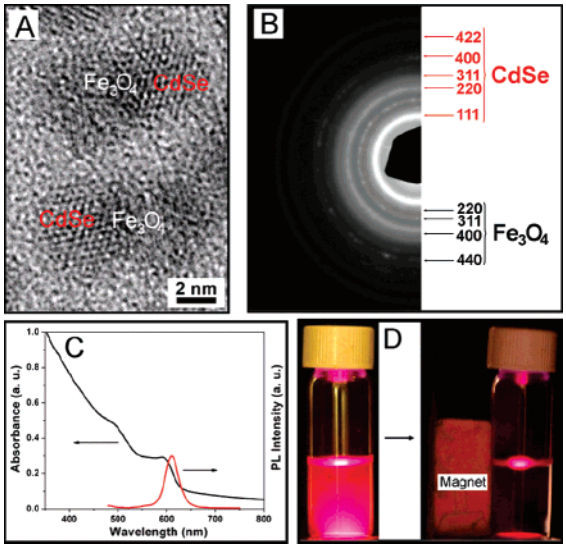
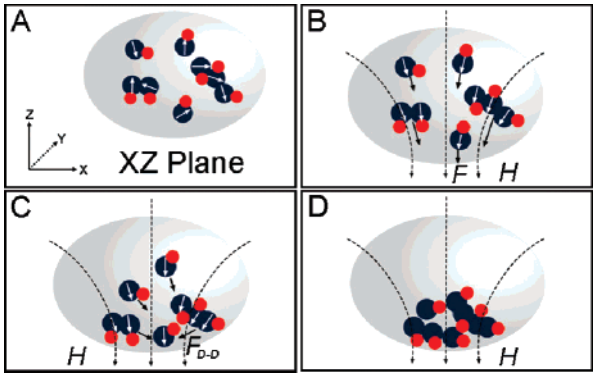


Figure 1. (A) An HRTEM image and (B) an EDP analysis of Fe_3O_4−CdSe heterodimers. (C) The UV−vis and fluorescence spectra of Fe_3O_4−CdSe nanoparticles in hexane solution. (D) The fluorescence images of the hexane solution of Fe_3O_4−CdSe nanoparticles before and after attraction by a small magnet (excited by a UV lamp, $\lambda_{ex} = 365$ nm).

Scheme 1



As expected, the Fe_3O_4−CdSe nanoparticles exhibit both fluorescence and superparamagnetism. A UV−visible peak at about 600 nm (Figure 1C) corresponds to the absorption of the CdSe part in Fe_3O_4−CdSe nanoparticles. The emission peak of the nanoparticles at 610 nm has a narrow full width at half-maximum (fwhm $\approx$ 30 nm) without significant fluorescent quenching (*quantum yield* $\approx$ *38%*),[8] possibly due to the introduction of metal oxide instead of metal.[9] The shapes of the photoluminescence excitation (PLE) spectra[8] of the nanoparticles are similar to the shape of the UV−vis spectrum, suggesting that the CdSe domain behaves as an

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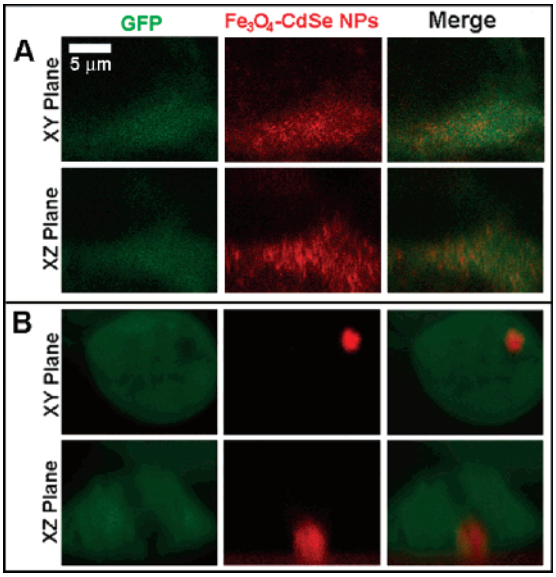

Figure 2. After the HEK293T cells are incubated with the Fe_3O_4−CdSe@GSH nanoparticles and pEGFP-N1 vector, these confocal images were taken of the cell (A) without a magnetic field (homogeneous fluorescent spots suggesting that the nanoparticles mainly distribute in the cytosol) and (B) under a magnetic field for 8 h.

individual quantum dot. Standard zero-field cooling (ZFC) and field cooling (FC) measurements yield the estimated blocking temperature of ≈75 K for the Fe_3O_4−CdSe nanoparticles,[8] suggesting that the magnetic behavior of the heterodimers originates from the Fe_3O_4 parts. Because of the magnetic moments of Fe_3O_4, the small magnet attracts the fluorescent Fe_3O_4−CdSe nanoparticles completely to the side of a vial after 10 min, and only a clear solution remains (Figure 1D).

After glutathione (GSH) molecules modify the surface of the particles,[10] the largest hydrodynamic radius measured fits the clusters of three Fe_3O_4−CdSe nanoparticles, suggesting that the nanoparticles disperse well in water.[8] At room temperature, the magnetic moments of Fe_3O_4−CdSe nanoparticles and Fe_3O_4−CdSe@GSH nanoparticles are ≈3.2 emu/g and ≈1.1 emu/g, respectively,[8] which are sufficient to allow the nanoparticles to be influenced by a small magnet with a surface magnetic field of ∼3000 G.[8] The GSH-modified nanoparticles and pEGFP-N1 vector (for the expression of green fluorescent protein (GFP)) were incubated with HEK293T cells for the imaging experiments.[8] After 24 h of incubation, the incubation medium was replaced, and the cells were washed in a fresh DMEM medium before being placed on a glass slide. Then, a small magnet was fixed under the slide to attract the fluorescent magnetic nanoparticles to one side of the cells. Using a confocal microscope, we captured fluorescent images in the XY plane and XZ plane to examine the movement of Fe_3O_4−CdSe@GSH nanoparticles inside the cells before and after applying the magnet. Figure 2A shows the confocal images of the cells without the application of a magnetic force, indicating that the image from CdSe in Fe_3O_4−CdSe@GSH matches with the image from GFP and confirming that the Fe_3O_4−CdSe@GSH particles distribute nonspecifically inside the cells. After being attracted by a small magnet, the Fe_3O_4−CdSe@GSH nanoparticles or the clusters aggregate on the side of the cell nearest the magnet, as shown in Figure 2B. Because of the significant magnetic moment of the Fe_3O_4−CdSe@GSH nanoparticles, the nanoparticles or their clusters drift to the magnet due to the magnetic field gradient,[11] as illustrated in Scheme 1. When the nanoparticles or the clusters approach each other, the magnetic dipolar−dipolar interactions (F_{D-D}) become the dominant forces and drive the aggregates of nanoparticles closer to the magnet. The interplay of these two forces leads to the result of round aggregates of the nanoparticles inside the cells. Although the high viscosity of the cytosol partially hindered the movement of Fe_3O_4−CdSe@GSH nanoparticles inside the cells and resulted in a slow response, almost all of the nanoparticles moved toward the small magnet and were located on the one side of the cells after 8 h (Figure 2B).[8]

In summary, we showed the first intracellular manipulation of fluorescent magnetic nanoparticles using a small magnet. Although the antibody-targeted specific location of nanoparticles in cells has been realized,[12] the intracellular manipulation of nanoparticles is less developed. The strategy demonstrated here may offer a useful tool to investigate the difference between the apical and basolateral domains in polarized cells by the asymmetric localization of magnetic nanoparticles that carry specific ligands. In addition, the successful intracellular control of magnetic nanoparticles may open up other new biological applications of magnetic nanoparticles because the principles of this work should be useful to the applications of other multifunctional magnetic nanoparticles.

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Supporting Information Available: Experimental section, EDS, PLE, magnetic measurements, optical images, and confocal images. This material is available free of charge via the Internet at http://pubs.acs.org.

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